PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Baldwin Shelston Waters
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SYDNEY NSW 2000

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *day/month/year* 25 NOV 2003
Applicant's or agent's file reference 32313WOP00	**IMPORTANT NOTIFICATION**

International Application No.	International Filing Date	Priority Date
PCT/AU02/01159	27 August 2002	27 August 2001

Applicant

PROTIGENE PTY LTD et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	ANDREW BRYCE Telephone No. (02) 6283 2263

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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 32313WOP00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU02/01159**	International Filing Date *(day/month/year)* 27 August 2002	Priority Date *(day/month/year)* 27 August 2001
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C07K 1/14, 1/36, 19/00; C12N 9/00, 15/63		
Applicant PROTIGENE PTY LTD et al		

This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 11 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
18 March 2003	11 November 2003
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ANDREW BRYCE** Telephone No. (02) 6283 2263

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU02/01159

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-4, 6, 8-46,** as originally filed,
pages , filed with the demand,
pages **5, 7, 7a,** received on **30 October 2003** with the letter of 30 October 2003

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **47-54,** received on **30 October 2003** with the letter of **30 October 2003**

☒ the drawings, pages **1/9-9/9,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU02/01159

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-70**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-70**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-70**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

The report is based on the following documents, as cited in the ISR:

D1: Westphal, M. *et al.* (1997) Current Biology 7 (3): 176-183
D2: Coen, L. *et al.* (2001) Proc. Natl. Acad. Sci. USA 98 (14): 7869-7874
D3: Sun, Q. *et al.* (1998) J. Bacteriol. 180 (8): 2050-2056
D4: Hollomon, D. W. *et al.* (1998) Antimicrob. Agents Chemother. 42 (9): 2171-2173

D1 discloses a GFP-(linker)-actin fusion protein (see Figure 1), where actin is a self-polymerisable polypeptide. The linker used in this fusion protein does not comprise a protease recognition sequence, and D1 is not directed to a process of sequestering or purifying a protein of interest by using a polymerisable protein, therefore Claims 1-70 are novel and inventive over document D1.

D2 discloses a protein which can form heterodimers (Bcl-X_L/xR11 see page 7869, column 2, paragraph 2), and a GFP-xR11 fusion protein is disclosed, under the control of a β-tubulin promoter. This does not constitute a fusion protein with a polymerisable protein as defined in the present claims, therefore Claims 1-70 are novel and inventive over D2.

D3 discloses a FtsZ-GFP fusion protein (see page 2050, column 2, "Bacterial strains and culture conditions"), where FtsZ is an *E. coli* protein that is self-polymerisable polypeptide. This document does not use a linker polypeptide and is not directed to a process of sequestering or purifying a protein of interest by using a polymerisable protein. Therefore, Claims 1-70 are novel and inventive over D3.

D4 discloses a β-tubulin-maltose binding protein (MBP) fusion protein (see page 2171, column 2, last paragraph), where β-tubulin is a self-polymerisable polypeptide. This document does not use a linker polypeptide and is not directed to a process of sequestering or purifying a protein of interest by using a polymerisable protein. Therefore, Claims 1-70 are novel and inventive over D4.

The claims are considered to have industrial applicability.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A hybrid polypeptide comprising a polypeptide of interest linked to a polymerisable polypeptide by a linker polypeptide, wherein the linker polypeptide comprises a recognition site for a proteolytic agent.

2. A hybrid polypeptide according to claim 1 wherein the proteolytic agent is selected from the following group: 3C-protease from a human rhinovirus type 14 (HRV protease 3C), thrombin, Factor Xa, enterokinase and a chemical capable of proteolytic activity.

3. A hybrid polypeptide according to claim 2 wherein the proteolytic agent is 3C-protease from a human rhinovirus type 14 (HRV protease 3C).

4. A hybrid polypeptide according to claim 1 wherein the recognition site comprises an amino acid sequence selected from the following group: Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro, Leu-Val-Pro-Arg-Gly-Ser, Ile-Glu-Gly-Arg and Asp-Asp-Asp-Asp-Lys.

5. A hybrid polypeptide according to claim 2 wherein the chemical capable of proteolytic activity is cyanogen bromide.

6. A hybrid polypeptide according to any one of claims 1 to 5 wherein the linker polypeptide is encoded by a polynucleotide comprising a cloning site.

7. A hybrid polypeptide according to claim 6 wherein the cloning site is a multiple cloning site.

8. A hybrid polypeptide according to any one of claims 1 to 7 wherein the linker polypeptide comprises a spacer polypeptide of sufficient length to allow or enhance cleavage of the polypeptide of interest from the polymerisable polypeptide, or to avoid

unfavourable steric interference between the polypeptide of interest and the polymerisable polypeptide.

9. A hybrid polypeptide according to claim 1 wherein the polypeptides are linked by antibody interaction.

10. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved by a process comprising attaching an antibody specific for the polymerisable polypeptide to the polypeptide of interest.

11. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved by a process comprising attaching an antibody specific for the polypeptide of interest to the polymerisable polypeptide.

12. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved using a bi-specific antibody directed to both the polypeptide of interest and the polymerisable polypeptide.

13. A hybrid polypeptide according to any one of claims 1 to 12 wherein the polymerisable polypeptide is a polypeptide that naturally polymerises with itself.

14. A hybrid polypeptide according to claim 13 the polymerisable polypeptide is tubulin or actin.

15. A hybrid polypeptide according to claim 13 wherein the polymerisable polypeptide is an FtsZ protein or a variant thereof.

16. A hybrid polypeptide according to claim 15 wherein the polymerisable peptide is *E. coli* FtsZ protein or a variant thereof.

17. A hybrid polypeptide according to claim 16 wherein the variant *E. coli* FtsZ protein comprises replacement of the aspartate residue at position 212 of the protein with a cysteine or asparagine residue.

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18. A hybrid polypeptide according to claim 16 wherein the variant FtsZ comprises a mutation selected from one of the following: replacement of alanine by threonine at position 70, replacement of aspartate by alanine at position 209 and replacement of aspartate by alanine at position 269.

19. A hybrid polypeptide according to any one of claims 1 to 18 wherein the polymerisable polypeptide requires an intermediary polypeptide or other molecule in order to polymerise.

20. A hybrid polypeptide according to any one of claims 1 to 19 wherein the polypeptide of interest is of prokaryotic origin.

21. A hybrid polypeptide according to any one of claims 1 to 19 wherein the polypeptide of interest is of eukaryotic origin.

22. A hybrid polypeptide according to any one of claims 1 to 21 wherein the polypeptide of interest is selected from the group comprising: an endonuclease, a methylase, an oxidoreductase, a transferase, a hydrolase, a lysase, an isomerase, a ligase, a storage polypeptide, a ferritin, an ovalbumin, a transport protein, haemoglobin, serum albumin or ceruloplasmin, an antigen, an antigenic determinant for use in the preparation of vaccines or diagnostic agents, a protective protein, a defence protein, thrombin, fibrinogen, binding proteins, antibodies, immunoglobulins, a human growth hormone, somatostatin, prolactin, estrone, progesterone, melanocyte, thyrotropin, calcitonin, gonadotropin, insulin, a hormone identified as being involved in the immune system, interleukin 1, interleukin 2, colony simulating factor, macrophage-activating factor, interferon, a structural element, collagen, elastin, alpha-keratin, glyco-protein, virus-protein and muca-protein.

23. A hybrid polypeptide according to claim 22 wherein the polypeptide of interest is a protease.

24. A hybrid polypeptide according to claim 23 wherein the protease is 3C-protease from human rhinovirus type 14 (HRV protease 3C).

25. A hybrid polypeptide according to any one of claims 1 to 23 wherein the polypeptide of interest is a synthetic polypeptide.

26. A method of sequestering and/or purifying a polypeptide of interest comprising the step of polymerising a hybrid polypeptide which hybrid polypeptide comprises the polypeptide of interest linked to a polymerisable polypeptide.

27. A method according to claim 26 wherein the polypeptide of interest is linked to the polymerisable polypeptide by fusing the polypeptide of interest directly to the polymerisable polypeptide.

28. A method according to claim 26 wherein the polypeptide of interest is linked to the polymerisable polypeptide by a linker polypeptide.

29. A method according to any one of claims 26 to 28 wherein the hybrid polypeptide is produced *in vivo*.

30. A method according to claim 26 wherein the hybrid polypeptide is a polypeptide according to any one of claims 1 to 25.

31. A method according to any one of claims 26 to 30 wherein polymerisation is performed under controlled chemical and/or physical conditions.

32. A method according to any one of claims 26 to 31 wherein the polymerisable polypeptide is polymerised by the addition of an agent which induces polymerisation.

33. A method according to claim 32 wherein the polymerisation inducing agent is GTP, ATP and/or a cation.

34. A method according to claim 33 wherein the cation is selected from the following group: magnesium, calcium, nickel, cobalt, zinc and manganese.

35. A method according to claim 31 wherein the polymerisable polypeptide is polymerised by a change in temperature.

36. A method according to any one of claims 26 to 35 wherein the polymerised hybrid polypeptide is purified by a first purification step and wherein the first purification step may be the only purification step or may be followed by further purification steps.

37. A method according to claim 36 wherein the first purification step purifies the polymerised hybrid polypeptide by physical techniques discriminating on the basis of size and/or weight.

38. A method according to claim 37 wherein the polymerised hybrid polypeptide is purified by centrifugation, differential sedimentation, filtration, dialysis and/or flow sorting such that the polymerised hybrid polypeptide is isolated.

39. A method according to claim 38 wherein after the first purification step the polymerised hybrid polypeptide is dissociated.

40. A method according to claim 39 wherein dissociation is achieved by removal of the agent which induces polymerisation and/or incubation of the polymerised hybrid polypeptide at a suitable temperature.

41. A method according to claim 39 or claim 40 wherein the dissociated hybrid polypeptide is purified by a second purification step.

42. A method according to claim 41 wherein the second purification step comprises purification of the hybrid polypeptide on the basis of size and/or weight.

43. A method according to claim 41 wherein polymerisation, dissociation and purification of the polymerisable hybrid polypeptide are repeated such that substances larger and smaller than the hybrid polypeptide are removed.

44. A method according to any one of claims 26 to 43 wherein the polymerisable polypeptide is cleaved from the polypeptide of interest by a protease.

45. A method according to claim 44 wherein cleavage by the proteolytic agent does not substantially interfere with the biological or chemical activity of the polypeptide of interest or the polymerisable polypeptide.

46. A method according to claim 44 or claim 45 wherein the proteolytic agent is a protease.

47. A method according to claim 46 wherein the protease is linked to a polymerisable polypeptide to form a "protease hybrid polypeptide".

48. A method according to claim 47 wherein the polymerisable polypeptide to which the protease is linked is identical to the polymerisable polypeptide to which the polypeptide of interest is linked, or is a variant thereof.

49. A method according to claim 47 or claim 48 wherein after cleavage of the polypeptide of interest from the polymerisable polypeptide, the protease hybrid polypeptide is polymerised.

50. A method according to claim 49 wherein the polypeptide of interest is purified from the polymerised protease hybrid polypeptide.

51. A method according to any one of claims 44 to 46 wherein the proteolytic agent is fused to the hybrid polypeptide.

52. A method according to any one of claims 44 to 51 wherein the polymerisable polypeptide released after cleavage from the polypeptide of interest is polymerised.

53. A method according to claim 52 wherein the polymerised polymerisable polypeptide is removed from the polypeptide of interest by a method which discriminates on the basis of size and/or weight.

54. A method according to any one of claims 46 to 53 wherein the protease is 3C-protease from a human rhinovirustype 14 (HV protease 3C).

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55. A method according to any one of claims 26 to 54 wherein the hybrid polypeptide is linked to a support.

56. A method according to claim 55 wherein the support comprises a polymerisable polypeptide.

57. A method according to claim 56 wherein the support polymerisable polypeptide comprises a polymerisable polypeptide identical to the hybrid polypeptide, or a variant thereof.

58. A hybrid nucleic acid comprising a nucleic acid encoding a hybrid polypeptide according to any one of claims 1 to 25.

59. A library comprising a plurality of hybrid nucleic acids according to claim 58.

60. A vector comprising a hybrid nucleic acid according to claim 58.

61. A library of vectors comprising vectors according to claim 60.

62. A cell transformed or transfected with a hybrid nucleic acid according to claim 58, a library according to claim 59, a vector according to claim 60, or a library of vectors according to claim 61.

63. Cells transformed or transfected with a library according to claim 59 or 61.

64. A library comprising a plurality of hybrid polypeptides according to any one of claims 1 to 25.

65. Use of a hybrid nucleic acid according to claim 58, a library according to claim 59, a vector according to claim 60, or a library of vectors according to claim 61 in a method of sequestering and/or purifying a polypeptide of interest.

66. A polypeptide of interest when purified by a method according to any one of claims 26 to 57.

67. A library of polypeptides of interest according to claim 66.

68. A method of purifying a polypeptide of interest comprisi

(a) expressing the hybrid nucleic acid of claim 58 in a cell to produce a hybrid polypeptide comprising the polypeptide of interest and a polymerisable polypeptide;

(b) polymerising the hybrid polypeptide;

(c) purifying the polymerised hybrid polypeptide;

(d) cleaving the polypeptide of interest from the polymerisable polypeptide; and

(e) purifying the polypeptide of interest.

69. A method according to claim 68 wherein the polypeptide of interest is cleaved from the polymerisable polypeptide by a protease which protease is itself linked to a polymerisable polypeptide to form a protease hybrid polypeptide.

70. A method according to claim 69 wherein after cleavage, the polymerisable polypeptide linked to the protease is polymerised and the polypeptide of interest is purified by removal of the polymerised protease hybrid polypeptide.